**BACTERIAL DILUTIONS and A FOOL-PROOF WAY TO FIGURE THEM OUT**

Look at the dilution scheme below:

Most questions you will be asked to answer about serial dilutions are of two types:

The **FIRST TYPE** gives you the number of bacterial colonies found on a plate and asks for the number of bacteria per ml in the original culture.

The **SECOND TYPE** gives you the number of bacteria per ml in the original culture and asks you to devise a serial dilution scheme so that you will get plates with “countable” numbers (i.e., between 30 and 300 colonies) of colonies on them.

To solve **TYPE ONE** problems, first determine the individual dilution factor for each tube using the formula:

\[
\text{INDIVIDUAL DILUTION FACTOR} = \frac{\text{AMOUNT TRANSFERRED}}{\text{AMOUNT TRANSFERRED} + \text{AMOUNT ALREADY IN TUBE}}
\]

For Tube A, the IDF = \( \frac{0.1}{0.1 + 9.9} = 0.01 = 10^{-2} \)

For Tube B, the IDF = \( \frac{0.5}{0.5 + 4.5} = 0.1 = 10^{-1} \)

For Tube C, the IDF = \( \frac{0.01}{0.01 + 9.99} = 0.001 = 10^{-3} \)

For Tube D, the IDF = \( \frac{1.0}{1.0 + 9.0} = 0.1 = 10^{-1} \)

Next determine the total dilution factor for the entire dilution series using the formula:

\[
\text{TOTAL DILUTION FACTOR} = (\text{IDF}_A)(\text{IDF}_B)(\text{IDF}_C)(\text{IDF}_D)
\]
For the dilution series above, the TDF for tube A = 10^{-2}

The TDF for Tube B = (10^{-2})(10^{-1}) = 10^{-3}

The TDF for Tube C = (10^{-2})(10^{-1})(10^{-3}) = 10^{-6}

The TDF for Tube D = (10^{-2})(10^{-1})(10^{-3}) = 10^{-7}

We can assume that each colony of bacteria arose from one living (or viable) cell immobilized on an agar plate. Thus each colony is a clone of cells. We can now determine the number of live bacteria (or Colony Forming Units [CFU]) per ml of original culture being using the formula:

\[
\text{CFU/ml = number of colonies per ml plated} \quad \frac{\text{Total dilution factor}}{}
\]

As plate E has 275 colonies, in the original culture:

The CFU/ml = (275 colonies/ml plated) = 275 x 10^7 = 2.8 x 10^9 CFU/ml

Plate F has 28 colonies, but only 0.1 ml was plated:

The CFU/ml = (28 colonies/0.1 ml plated) = 280 x 10^7 = 2.8 x 10^9 CFU/ml

*** If you use these two formulae, you can solve any serial dilution problem.

To solve **TYPE TWO** problems, simply rearrange the formula above to solve for the total dilution factor:

\[
\text{TOTAL DILUTION FACTOR} = \frac{\text{NUMBER OF COLONIES/ML PLATED}}{\text{CFU/ML}}
\]

For example, if you want to have a plate with approximately 30 colonies on it and the original culture contains 2.8 x 10^9 CFU/ml, plug these values into the rearranged equation:

\[
\frac{30}{2.8 \times 10^9} = 1 \times 10^{-8}
\]

An easy way to set up dilution series like this would be to use 4 tubes, each having an IDF of 10^{-2}, i.e., transfer 0.1 ml into a tube containing 9.9 ml four times. Spread 1.0 ml on a plate and incubate.

**SELF TEST**

** How many colonies would you expect if you plated our 0.1 ml form Tube C?

** How many colonies would you expect if you plated out 1.0 ml from Tube C?

** IF the IDF for Tube A was 10^{-3} and the IDF for Tube B was 10^{-2}, what would be the TDF for Tube D?

** Starting with a culture that contains 3 x 10^8 CFU/ml, devise a serial dilution scheme that would yield a plate with 120 colonies.